University of Idaho
College of Agricultural and Life Sciences

Pre-release studies on the environmental safety of *Mogulones cruciger,* a root-mining weevil for the biological control of houndstongue in Idaho

Annual Report

Report period: 1 January – 31 December 2003

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Funded by **Idaho State Department of Agriculture, 2003 Cost-Share Agreement**
Big Horn Drainage Weed and Pest Districts, Wyoming

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Summary

1. The year 2003 was very successful for the houndstongue biocontrol research efforts at the University of Idaho. With funding from ISDA and the Big Horn Drainage Weed and Pest Districts in Wyoming we could continue our efforts to conduct research on the environmental safety of the houndstongue root weevil, which has already become a very successful biological control agent in parts of Alberta and British Columbia.

2. During the 2003 Annual Meeting of the Technical Advisory Group (TAG) organized by Dr. Linda Wilson and Schwarzlaender in Spokane it became clear that USDA APHIS will not issue permission for release of this agent in the U.S. unless additional data on the environmental safety of this biocontrol agent becomes available and an additional petition is issued. The University of Idaho took on the task to provide this research data.

3. Non-target attack monitoring was continued in 2003 at houndstongue root weevil release sites in southern Alberta and southern British Columbia. Non-target attack was found but it was inconsistent at different sites. Non-target attack was low and usually occurred at high weevil densities, an indication for so called 'spill-over' effects: Non-target plant species are only fed upon when houndstongue plants become rare and weevils accept alternative low-quality host plants. Spill-over effects also predict that in the following year the attack will disappear because non-target plants cannot sustain survival of the houndstongue root weevil.

4. During 2003, we successfully obtained plant material of threatened or endangered (T or E) listed Boraginaceae species. We started to conduct host range experiments with the weevils from our quarantine rearing with T or E listed non-target plants. Thus far, our results are promising and we plant to continue these tests in early 2004.

5. More data on the experimental host range (in regard to rare Boraginaceae) and the realized host range (in regard to non-target attack at field sites in Canada) is needed before we can conclusively summarize the environmental safety of the weevil in a petition to TAG.

1 Introduction

Houndstongue, *Cynoglossum officinale* L. (Boraginaceae), is a noxious short-lived perennial weed of mountainous rangelands in northwestern North America. Native to Europe, the weed was likely introduced as a contaminant of cereal in the 1800s and has since spread throughout Canada and the continental United States. It is particularly prevalent in the southern interior of British Columbia, northern Wyoming, eastern Montana, western Washington and all parts of Idaho (Fig. 1).

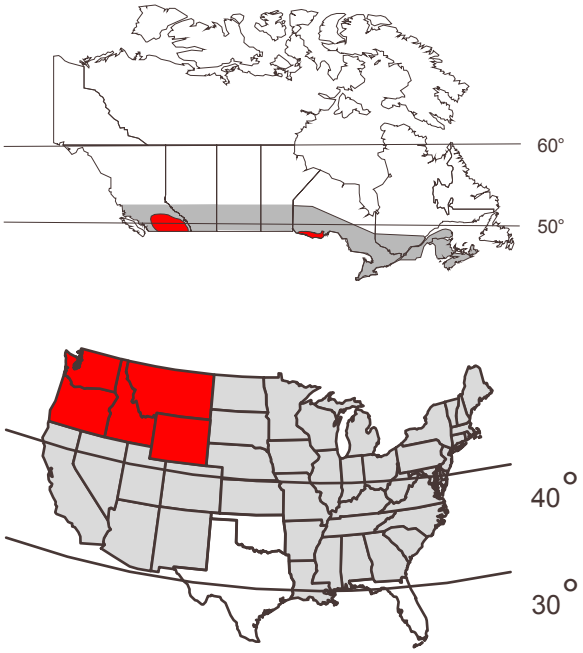

Fig. 1 Distribution of *Cynoglossum officinale* L. in North America. Red, prevalent; grey, present.

Cattle producers in these areas consider houndstongue to be one of the most serious rangeland weed problems because of its burrs and toxicity. The seeds are borne in burred nutlets that attach to livestock hair, thus causing irritation and potential reductions in livestock price (Fig. 2). Houndstongue is highly toxic to cattle and horses and deaths have been reported. The toxicity of *C. officinale* is related to pyrrolizidine alkaloids (PAs), which occur at levels much higher than those found in most other toxic rangeland weeds. Livestock avoid feeding on green houndstongue, but problems arise when the plants senesces or is dried in hay. Calves fed 1 kg of dried plant per kg of body weight died within 48 hours due to severe liver damage and one quarter of this dose cause eventual death.

Fig. 2 Cattle can become covered in seeds, which they spread by rubbing them off in different areas.

A biological control program to control the weed was initiated in 1988. Five biocontrol agents were or are being investigated in Europe for their potential to control houndstongue. Foreign exploration and testing of two potential biocontrol agents is underway and a third insect species, the houndstongue seed weevil will undergo final testing at the CABI Bioscience Switzerland Center and the University of Idaho during spring 2004. The remaining two species, the root-mining flea beetle *Longitarsus quadriguttatus* and the houndstongue root weevil *Mogulones cruciger* were first release in Canada in 1998 and 1997, respectively. In 1999, *M. cruciger* was released at the Idaho-Alberta border nearby Kingsport/Eastport. *M. cruciger* is a root-feeding weevil which at high infestation rates can kill houndstongue plants. In Canada, the weevil has established at many field sites, is increasing in numbers, and dispersing to new sites. *M. cruciger* successfully controls houndstongue populations already at various locations (Fig. 3). In outdoor propagation plots at Lethbridge, AB, and Kamloops, BC, the weevil has shown an excellent capacity for population increase and impact, to the extent that it is now difficult to keep houndstongue plants available.

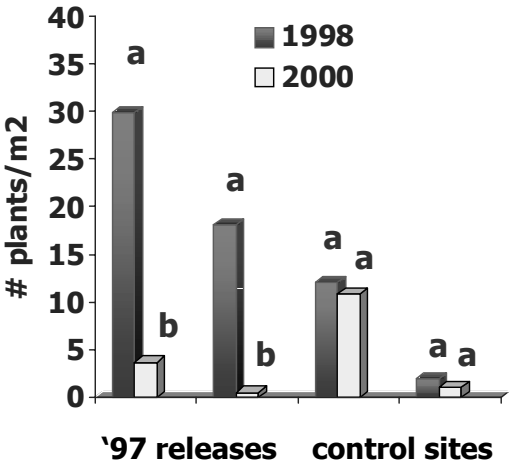

Fig. 3 Impact of *Mogulones cruciger* on houndstongue populations in British Columbia. Dark bars, plant densities at the time of release in 1997 at two release sites (left) and two corresponding control sites (right); light bars, plant densities in 2000 at the four sites (Different letters on top of bars represent significant differences; Oneway ANOVA; $P < 0.001$, n = 20). R. DeClerck-Floate, AAFC; Unpublished data.

The experimental host range of the houndstongue root weevil (Fig. 4) was assessed between 1988 and 1996 and a petition for field release was submitted to the Technical Advisory Group (TAG), a federal interagency expert committee charged with the introduction of exotic beneficial organisms. TAG recommended the release of the weevil release in North America. The weevil was subsequently approved for release in Canada by the Canadian food Inspection Agency and first releases were made in 1997. The permission for field release in the United States is still pending due to concerns of the USDA APHIS PPQ and the U.S. Fish and Wildlife Service about the environmental safety of the weevil.

Fig. 4 *M. cruciger* adult (left) and larval feeding damage (right).

There is particular concern that non-target species in the family Boragincaeae are attacked by the weevil that are federally listed as threatened or endangered: *Hackelia venusta* (Piper) St. John, *Amsinckia grandiflora* (Kleeb. ex Gray), *Cryptantha crassipes* I.M. Johnston), and *Plagiobothrys hirtus* (Greene) I.M. Johnston). None of these species was included in previous host-specificity tests. Therefore, we do not know whether the weevil poses a risk to those or other rare Boraginaceae that occur in the Pacific Northwest. It is currently not possible to release or distribute *M. cruciger* in the U.S. The weevil might soon enter the U.S. accidentally. However, new agricultural anti-terrorism policies of USDA APHIS PPQ will restrict movement of non-authorized organisms between *and within* states.

We propose to study the environmental safety of *M. cruciger* in Idaho prior to potential mass rearing and redistribution programs. With the proposed research, we hope to demonstrate that *M. cruciger* has no significant non-target effects on native Boraginaceae plant species that are rare, threatened, or endangered. We hope that the results of our research will lead to the issuance of permission for field release of the houndstongue root weevil in the U.S. We expect that *M. cruciger* will have a significant impact on the reduction of houndstongue populations in Idaho once released.

2 Work during 2003

Funds received from ISDA and Big Horn Drainage Weed and Pest Districts, Wyoming in 2003 were used to continue studies on the realized host range (at Canadian field site) and the experimental host range (laboratory tests in quarantine) of the houndstongue root weevil.

Realized host range at field sites in Canada: Plants were collected from four sites in Alberta and two in British Columbia during June 2003. Each of the sites had relatively low populations of houndstongue and two or more co-occurring native North American Boraginaceae species. The native plant species included *Hackelia floribunda* (Lehm.) I.M. Johnston, *Lithospermum ruderale* Dougl. ex

Lehm., *Lappula squarrosa* (Retz.) Dumort. and *Cryptantha celosioides* Lehm. ex G. Don. In 2000, four hundred weevils were initially released at each of these field sites. Initial monitoring was conducted in August 2001 by harvesting approximately 10 individuals of each plant species at random and dissections of the roots for *M. cruciger* eggs or larvae. A small proportion of larvae were found in roots of houndstongue but and no non-target species showed signs of attack. In April 2002, we augmented the present *M. cruciger* populations by releasing another 200 weevils at each site. We wanted to increase the weevil density and thus, the probability of non-target effects. In late June 2002, *C. officinale* and non-target species were again sampled and roots were dissected for *M. cruciger* eggs and larvae. In 2003, we continued to plant sampling using a modified T-square sampling method, which allowed us to collect a pairs of houndstongue and non-target plants. Approximately 15 houndstongue/non-target plant pairs were collected per non-target species. However, the exact number of harvested plants was largely determined by the abundance of each species at each site. This sampling method was chosen because it is particularly suitable for aggregated plant distributions. We found non-target effects in 2003, however attack rates were low despite the highest weevil densities found since beginning of the monitoring (Fig. 5).

Each site appears to have varying degrees of insect densities and this may have contributed to the attack rates found on non-targets plants. The probability of non-target effects increases with the biocontrol agent attack rates. This phenomenon is called 'spill-over' effect or 'central exaltation'. It is assumed that a decline of the host weed first causes non-target effects but that with the disappearance of the weed, the non-target effects also disappear because non-target species usually cannot sustain biocontrol agent populations.

We would use 2004 funding to continue our non-target field monitoring in order to correlate weevil attack rates with on-target attack probability. We think the 2003 data is very promising. We hope to demonstrate that non-target effects occur at high biocontrol agent densities and subsequently disappear.

Experimental host range on rare and threatened Boraginaceae species under laboratory conditions in quarantine: Single-choice feeding and oviposition and single-choice larval development experiments were conducted in quarantine in early spring 2003. Host range tests were conducted with rare, threatened, or endangered (T or E) listed species under quarantine conditions to determine the physiological host range of *M. cruciger*, i.e. the range of plant species the weevil can (partly) develop when forced.

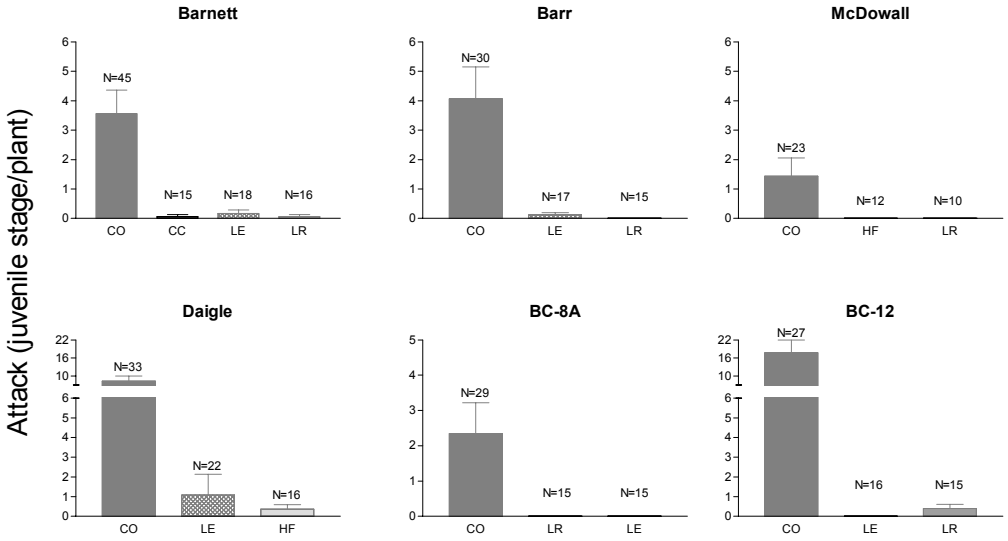

Fig. 5 Field collected non-target data in 2003 at 4 houndstongue field sites in Alberta and 2 in British Columbia. CO, *Cynoglossum officinale;* CC, *Cryptantha celosioides;* LE, *Lappula echinata;* LR, *Lithospermum ruderale ;* HF, *Hackelia floribunda.*

Plants were either collected in the field or grown from seed.

Plant material for T&E listed species was kindly provided by Dr. Sarah Reichard, University of Washington (*Hackelia venusta* (Piper) St. John), Dr. Tina Carlsen, Lawrence Livermore National Laboratory (*Amsinckia grandiflora* (Kleeb. ex Gray) Kleeb. ex Greene), Dr. John Gaskin, USDA-ARS (*Cryptantha crassipes* I.M. Johnston) and Dr. Rose DeClerck-Floate, Agriculture and Agri-Food Canada (*Plagiobothrys hirtus* (Greene) I.M. Johnston). Species used for tests also include but are not limited to *Dasynotus daubenmirei* I.M. Johnston, *Hackelia californica* (Gray) I.M. Johnston, *H. venusta*, *Mertensia. paniculata* (Ait.) G. Don, *Cryptantha crassipes*, *C. torreyana* (Gray) Greene, *Cryptantha celosioides*. (Eastw.) Payson and *Plagiobothrys hirtus* (Greene) I. M. Johnston. These species are all found in the Pacific Northwest with the exception of *C. crassipes,* which occurs in southwestern Texas, and *A. grandiflora* and *P. hirtus*, which occur in California. The latter three plant species are federally listed (T or E) species and require testing because of concerns of the U.S. Fish and Wildlife Service. Plants were grown from seed at the University of Idaho's Manis Entomology Laboratory greenhouse and moved to the Northwest Biological Control Insectary and Quarantine at Washington State University in Pullman, WA where tests were conducted. Single-choice adult feeding and oviposition tests and single-choice adult oviposition and larval development tests were conducted

1) Single-choice adult feeding and oviposition tests were conducted using stem cuttings from one test plant and one control (*C. officinale*) plant. Each cutting was inserted in a horticultural sponge block welded into plastic foil and placed in the opposite corners of plastic cages (25 cm width, 25 cm length, 50 cm height). The weevils (6 females and 4 males) were placed in an open petri dish in the centre of the cage and left to choose the preferred plant. Each replicate was set up for 3 days and tests were replicated 2-5 times for each test plant species. Data collected included the number of feeding holes (the average size of a feeding hole for *M. cruciger* is 4mm^2), the number of eggs laid, and the location of the weevils (test plant, control plant or cage) at the time the test was disassembled.

2) Single-choice oviposition and larval development tests were set up in the same fashion except that whole plants were used and they were tested in mesh cages (of similar size). Six gravid females and four males of *M. cruciger* were placed in an open petri dish into the centre of the cage floor. After 5 days, adults were retrieved and plants kept under growing lights to allow *M. cruciger* larval development. After 5 weeks, plants were dissected for mature larvae and soil sifted for pupae.

Generally, *M. cruciger* females showed a strong preference for the field host houndstongue during oviposition tests. Females did however, under the test conditions accept *Amsinckia grandiflora*, *Cynoglossum grande*, and *Mertensia paniculata* for oviposition to a limited extent (Fig. 6a).

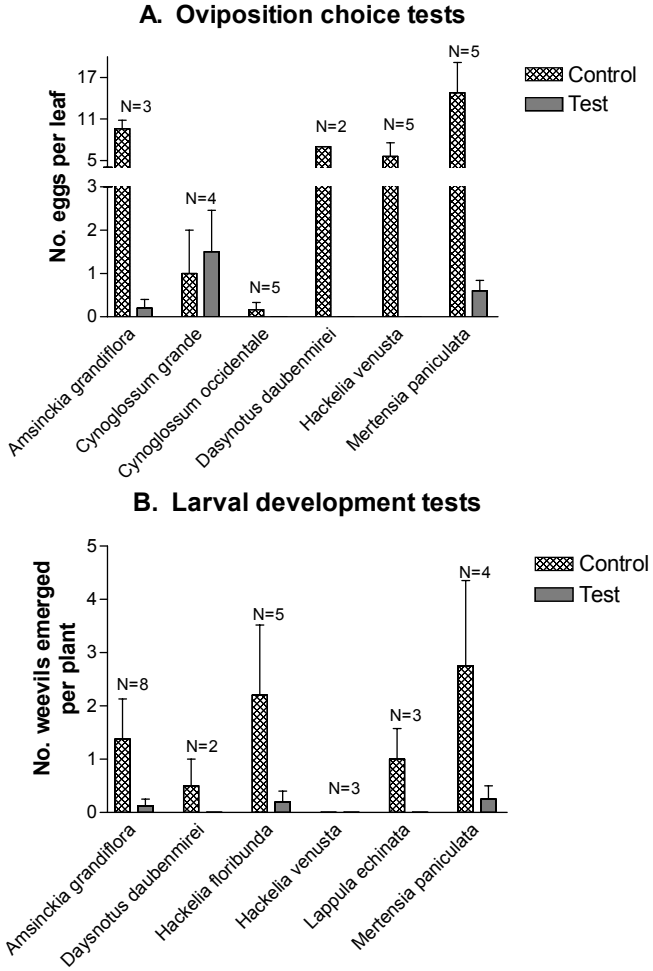

Fig. 6 Single-choice oviposition tests (A) and larval development tests (B) conducted in spring 2003 under quarantine conditions.

In larval developmental tests (Fig. 6b) the pattern of attack was similar. Houndstongue was preferably attacked. However, weevils emerged from *A. grandiflora*, *Hackelia floribunda* and *Mertensia paniculata* In general, fecundity rates of females were poor in both tests based on the attack rates found for houndstongue. We therefore, intend to repeat trials with these and additional non-target plant species in January 2004 and if funding becomes available in June 2004.

We were successful to acquire additional plant material for *Amsinckia grandiflora* and *Hackelia venusta*. In addition, we have finally successfully acquired *Plagiobothrys hirtus* and *Cryptantha crassipes* seeds. Therefore, we will be able to conduct tests with four of the five federally listed threatened or endangered Boraginaceae. Plants of the abovementioned species are currently grown for tests in greenhouses of the University of Idaho.

3 Conclusions and outlook

It took almost 2 years to establish contacts with botanists and ecologists to organize plat material T or E listed Boraginaceae. During that time, we also had to find a quarantine facility to conduct experiments and finally we had to obtain a permit for the introduction of the weevil into quarantine from USDA APHIS. Although this process was lengthy, we are at the point that we have an ongoing weevil rearing at the quarantine and the majority of the T or E and additional rare Boraginaceae of the Pacific Northwest growing in greenhouses at the University of Idaho. We also were able to select additional field sites in British Columbia and we fine-tuned the field monitoring methodology. In many respects, 2003 was the first field season during which we could collect quantitative data.

We think that both, the field and the laboratory data are promising. We are optimistic to demonstrate that non-target effects occur if at all only temporarily and only at high densities of the biocontrol agent and depleted houndstongue population levels. In addition, we think that we will be able to show with additional laboratory testing in the quarantine that the majority of rare, endangered, or threatened Boraginaceae species cannot be utilized as host plants by *M .cruciger*. We further think that we can conclusively show that even those plant species that to a certain extent could utilized by the weevil are not selected during the host plant selection process.